

WHAT IS CLAIMED:

1 1. An expression plasmid comprising an RNA polymerase I (pol I) promoter
2 and pol I terminator sequences, which are inserted between an RNA polymerase II (pol II)
3 promoter and a polyadenylation signal.

1 2. The expression plasmid of claim 1 wherein the pol I promoter is proximal
2 to the polyadenylation signal and the pol I terminator sequence is proximal to the pol II promoter.

1 3. The expression plasmid of claim 1 wherein the pol I promoter is proximal
2 to the pol II promoter and the pol I terminator sequence is proximal to the polyadenylation signal.

1 4. The expression plasmid of claim 1 wherein the plasmid corresponds to a
2 plasmid having a map selected from the group consisting of pHW2000, pHW11 and pHW12.

1 5. The expression plasmid of claim 1, further comprising a negative strand
2 RNA virus viral gene segment inserted between the pol I promoter and the termination signal.

1 6. The expression plasmid of claim 5, wherein the negative strand RNA virus
2 is a member of the *Orthomyxoviridae* virus family.

1 7. The expression plasmid of claim 6, wherein the virus is an influenza A
2 virus.

1 8. The expression plasmid of claim 7, wherein the viral gene segment encodes
2 a gene selected from the group consisting of a viral polymerase complex protein, M protein, and
3 NS protein; wherein the genes are derived from a strain well adapted to grow in cell culture or
4 from an attenuated strain, or both.

1 9. The expression plasmid of claim 6, wherein the virus is an influenza B
2 virus.

1 10. The expression plasmid of claim 8 wherein the plasmid has a map selected
2 from the group consisting of pHW241-PB2, pHW242-PB1, pHW243-PA, pHW245-NP,
3 pHW247-M, and pHW248-NS.

1 11. The expression plasmid of claim 8 wherein the plasmid has a map selected
2 from the group consisting of pHW181-PB2, pHW182-PB1, pHW183-PA, pHW185-NP,
3 pHW187-M, and pHW188-NS.

1 12. The expression plasmid of claim 7, wherein the viral gene segment encodes
2 a gene selected from the group consisting of an influenza hemagglutinin (HA) gene and a
3 neuraminidase (NA) gene.

1 13. The expression plasmid of claim 12, wherein the influenza gene is from a

1 pathogenic influenza virus strain.

1 14. The expression plasmid of claim 12, wherein the plasmid has a map
2 selected from the group consisting of pHW244-HA, pHW246-NA, pHW184-HA, and pHW186-
3 NA.

1 15. A minimum plasmid-based system for the generation of infectious negative
2 strand RNA viruses from cloned viral cDNA comprising a set of plasmids wherein each plasmid
3 comprises one autonomous viral genomic segment, and wherein the viral cDNA corresponding to
4 the autonomous viral genomic segment is inserted between an RNA polymerase I (pol I) promoter
5 and terminator sequences, thereby resulting in expression of vRNA, which are in turn inserted
6 between a RNA polymerase II (pol II) promoter and a polyadenylation signal, thereby resulting in
7 expression of viral mRNA.

1 16. The minimum plasmid-based system of claim 15 wherein the pol I
2 promoter is proximal to the polyadenylation signal and the pol I terminator sequence is proximal
3 to the pol II promoter.

1 17. The minimum plasmid-based system of claim 15 wherein the pol I
2 promoter is proximal to the pol II promoter and the pol I terminator sequence is proximal to the
3 polyadenylation signal.

1 18. The plasmid-based system of claim 15, wherein the negative strand RNA
2 virus is a member of the *Orthomyxoviridae* virus family.

1 19. The plasmid-based system of claim 18, wherein the virus is an influenza A
2 virus.

1 20. The plasmid-based system of claim 18, wherein the virus is an influenza B
2 virus.

1 21. The plasmid-based system of claim 19, wherein the viral gene segment
2 encodes a protein selected from the group consisting of a viral polymerase complex protein, an M
3 protein and an NS protein; wherein said genes are from a strain well adapted to grow in cell
4 culture or from an attenuated strain, or both.

1 22. The plasmid-based system of claim 19, wherein the viral genomic segments
2 comprise genes which encode a protein selected from the group consisting of hemagglutinin and
3 neuraminidase, or both; wherein said genes are from a pathogenic influenza virus.

1 23. The plasmid-based system of claim 19 wherein said system comprises one
2 or more plasmids having a map selected from the group consisting of pHW241-PB2, pHW242-
3 PB1, pHW243 -PA, pHW244-HA, pHW245-NP, pHW246-NA, pHW247-M, and pHW248-NS.

1 24. The plasmid-based system of claim 19, wherein said system comprises one
2 or more plasmids having a map selected from the group consisting of pHW181-PB2, pHW182-
3 PB1, pHW183 -PA, pHW184-HA, pHW185-NP, pHW186-NA, pHW187-M, and pHW188-NS.

1 25. A host cell comprising the plasmid-based system of claim 15.

1 26. A host cell comprising the plasmid-based system of claim 18.

1 27. A host cell comprising the plasmid-based system of claim 19.

1 28. A host cell comprising the plasmid-based system of claim 22.

1 29. A method for producing a negative strand RNA virus virion, which method
2 comprises culturing the host cell of claim 25 under conditions that permit production of viral
3 proteins and vRNA or cRNA.

1 30. A method for producing an *Orthomyxoviridae* virion, which method
2 comprises culturing the host cell of claim 26 under conditions that permit production of viral
3 proteins and vRNA or cRNA.

1 31. A method for producing an influenza virion, which method comprises
2 culturing the host cell of claim 27 under conditions that permit production of viral proteins and

1 vRNA or cRNA.

1 32. A method for producing a pathogenic influenza virion, which method
2 comprises culturing the host cell of claim 28 under conditions that permit production of viral
3 proteins and vRNA or cRNA.

1 33. A method for preparing a negative strand RNA virus-specific vaccine,
2 which method comprises purifying a virion produced by the method of claim 29.

1 34. The method according to claim 33, which further comprises inactivating the
2 virion.

1 35. The method according to claim 33, wherein the negative strand RNA virus
2 is an attenuated virus.

1 36. A method for vaccinating a subject against a negative strand RNA virus
2 infection, which method comprises administering a protective dose of a vaccine of claim 33 to the
3 subject.

1 37. A method for vaccinating a subject against a negative strand RNA virus
2 infection, which method comprises injecting a protective dose of a vaccine of claim 33
3 intramuscularly in the subject.

1 38. A method for vaccinating a subject against a negative strand RNA virus
2 infection, which method comprises administering a vaccine of claim 33 intranasally to the subject.

1 39. A method for generating an attenuated negative strand RNA virus, which
2 method comprises:

- 3 (a) mutating one or more viral genes in the plasmid-based system of claim 15;
4 and
5 (b) determining whether infectious RNA viruses produced by the system are
6 attenuated.

1 40. A composition comprising a negative strand RNA virus virion, wherein
2 viral internal proteins of the virion are from a virus strain well adapted to grow in culture or from
3 an attenuated strain, or both and viral antigen proteins, of the virion are from a pathogenic virus
4 strain.

1 41. A composition comprising a negative strand RNA virus virion produced by
2 the method of claim 29.